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[Article]

Actions of a New Cardiotonic Agent, SCH00013, on Guinea-pig Hearts

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Abstract

A new cardiotonic agent, SCH00013 (SCH), which was synthesized by Zenyaku Kogyo Company, Limited, exhibits cardiotonic effects by enhancing calcium sensitivity [1]. In this study, we examined the inotropic and chronotropic effects of SCH using right atrial muscles extirpated from guinea pigs. Although 10^{-4} M SCH showed positive inotropic effects (50% of those of noradrenaline (NAd)), there was no increase in the heart rate. In addition, we measured changes in the left ventricular diastolic pressure (LVDP) and transient Ca^{2+} (TCa) signals in Fura-2-loaded Langendorff hearts. The administration of 10^{-5} M SCH decreased the level of TCa, whereas 10^{-4} M SCH administration increased it. On the other hand, pimobendan (PIM), which is used as a drug for heart failure due to its calcium sensitivity-enhancing actions, decreased the level of TCa at 10^{-4} M despite an increase in contractility. This suggests that the mechanism of Ca^{2+} contractile protein system sensitivity differs between SCH and PIM. As SCH does not increase the heart rate in the presence of heart failure, it may protect the cardiac muscle.

Key words: SCH00013, positive inotropic effect (PIE), negative inotropic effects (NIE), positive chronotropic effect (PCE), heart rate (HR)

新規強心薬SCH00013のモルモット心臓に対する作用

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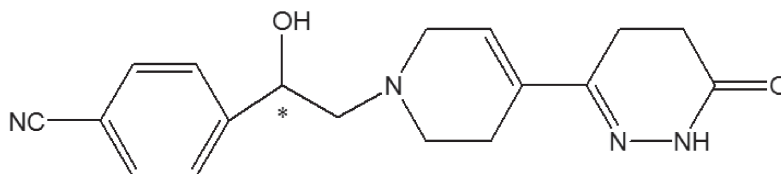
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1. Introduction

Cardiotonic effects mediated by calcium sensitivity-enhancing actions have the following merits: calcium mobilization-related myocardial energy may be saved without inducing the myocardial cell accumulation of calcium, and there is no risk of excessive calcium loading-related arrhythmia or myocardial cell disorder, differing from cardiotonic effects associated with receptor stimulation or phosphodiesterase inhibition. Pimobendan (PIM) is a representative, clinically available drug with calcium sensitivity-enhancing actions. It inhibits phosphodiesterase III (PDE-III) activity, and enhances Ca^{2+} sensitivity at a high concentration. Concerning cardiac muscle Ca^{2+} sensitivity-enhancing actions, cardiac contractility is increased without elevating the level of transient Ca^{2+} by enhancing the sensitivity of troponin C to Ca^{2+} [1]. Such actions are dose-dependent, and contractility is increased with low-level Ca^{2+} mobilization. Furthermore, a new cardiotonic agent, SCH00013 (SCH), which was synthesized by Zenyaku Kogyo Company, Limited, exhibits cardiotonic effects by enhancing calcium sensitivity [1].



SCH00013 (racemic mixture)

Fig. 1. Structural formula of a new cardiotonic drug, SCH00013 (SCH)

In this study, we examined the inotropic and chronotropic effects of SCH and PIM using right atrial muscles extirpated from guinea pigs. Although 10^{-4} M SCH showed PIE, there was no increase in chronotropic effects (heart rate). In addition, we simultaneously measured the left ventricular diastolic pressure (LVDP) and transient Ca^{2+} (TCa) signals in Fura-2-loaded Langendorff hearts. The SCH-related changes in Ca signals differed from those related to PIM, which is used as a drug for heart failure due to its calcium sensitivity-enhancing actions, or tea catechins (EGCg)[2] [3]. As SCH does not increase the heart rate, it may protect the cardiac muscle.

2. Materials and Methods

2.1 Experiment using isolated atrial specimens

Guinea pigs weighing 300 to 500g were sacrificed under ether anesthesia. Immediately, thoracotomy was performed, and the pulsating atrium was extirpated, and transiently placed in

nutrient solution to prepare a right atrial specimen. A pacemaker was stored in Krebs-Henseleit solution (30°C) saturated with mixed gas (95%O₂, 5%CO₂). Its end was fixed to a portion of a mixed gas influx tube in a sample tank with thread, and the other end was connected to the end of a tensiometer (Nihon Kohden Corp., TB-612T) with thread. Between the two points, 0.5 g resting tension was added. Autonomic pulsation-related tension was converted to voltage through a strain-gauge-type tensiometer, and recorded using a pen-type oscillograph (WATANABE INSTRUMENTS CORP., WR3101) through an amplifier (Nihon Kohden Corp., AP-601G, AT-601G).

2.2 Measurement of Fura-2 Ca²⁺ and NO signals in Langendorff (LN) hearts

1) Establishment of an NO electrode and Fura-2 loading:

A cannula was inserted into the aorta of the heart extirpated from a guinea pig (male, approximately 300 g), and Krebs-Henseleit solution (KH solution, Ca²⁺: 2 mM, pH: 7.4, 30°C, saturated with mixed gas consisting of 95%O₂ and 5%CO₂) was infused using a pump at a flow rate of 7 to 9 mL/min to prepare an LN heart. Concerning contractility, a balloon was inserted into the left ventricle, and the LVDP was measured. An NO-selective electrode was inserted into the right atrium to measure changes in NO outflow from the heart. While setting/stabilizing the NO electrode in the heart, KH solution containing 5μM fura-2 AM (25% Cremophore EL) was perfused for 30 minutes for heart loading with fura-2. Subsequently, the heart was washed with fresh KH solution to completely remove extracellular fura-2 AM, and stabilized for 20 minutes prior to this experiment.

2) NO measurement:

An improved electrode, consisting of NO-selective and control carbon electrodes, was inserted into the right atrium (coronary sinus), and NO measurement was performed under an electromagnetic wave-free environment; after removing noises on the reduction current (pA) of the NO-selective and control carbon electrodes through a 50/60 Hz noise eliminator (Hum Bug Quest Scientific Instrument, Canada), the level of NO was measured using an NO-meter (NO-501, Inter Medical Co.). A current value (pA) of which the basic value was stable on perfusion at a constant flow rate was regarded as zero [4].

3) Fluorescence measurement:

The Fura-2-loaded, extirpated heart was placed in the thermostat of the measurement unit of a fluorometer (CAF-110, JASCO) so that the left ventricular surface was exposed to light. While perfusing the heart with KH solution, the cardiac muscle was exposed to 340-and 380-nm exciting light, and the fluorescence intensity ratio (R340/380) at a fluorescence wavelength of 500 nm was recorded as changes in the intracardiac Ca²⁺ level [5]. The Fura-2 Ca²⁺ signals, NO signals, and LVDP were simultaneously recorded.

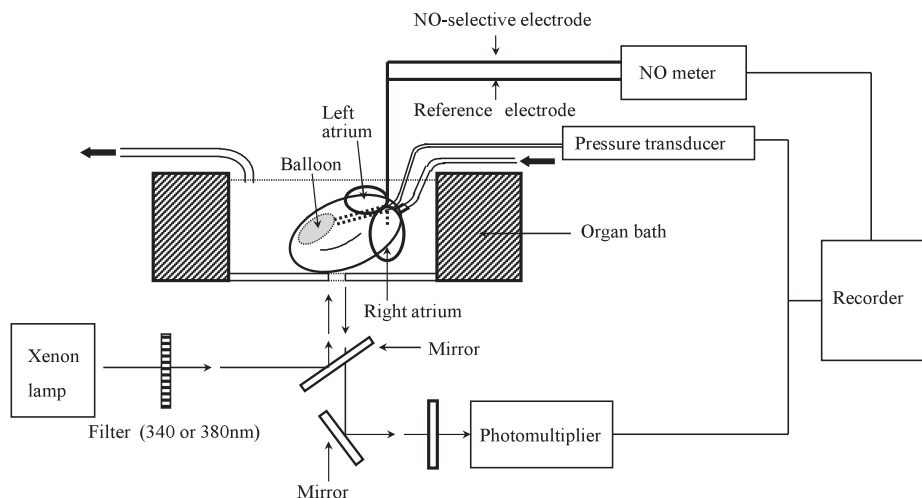


Fig. 2. CAF-110 device

3. Results

The cardiac contractility after SCH administration is shown in Fig. 3 (it was calculated, regarding a maximum at the time of 10^{-6} M noradrenaline (NAd) administration as 100%).

The dihydroouabain (DHO)-, PIM-, and SCH-related changes in the guinea pig right atrial contractility and heart rate are shown in Figs. 4(A), 4(B), and 4(C), respectively.

We compared cardiac contractility after DHO/PIM/SCH administration, regarding the value before the administration of each drug as 100%. All drugs showed PIE, but SCH more slowly increased contractility compared with PIM and DHO, and the incidence of arrhythmia at a high concentration was lower (Fig. 5(A)). The influence on the heart rate differed among the 3 drugs; SCH more markedly decreased the heart rate (HR) from the pretreatment value (100%) compared with PIM and DHO (Fig. 5(B)).

3.1 Actions on cardiac contractility

DHO showed slight PIE at 10^{-6} and 3×10^{-6} M, and cardiac contractility reached a maximum at 10^{-5} M. However, contracture gradually occurred at 10^{-5} M, and arrhythmia occurred at 3×10^{-5} M. NAd administration after the onset of arrhythmia did not lead to recovery (Fig. 4(A)). PIM exhibited PIE in a dose-dependent manner, and cardiac contractility reached a maximum at 3×10^{-5} M. In addition, it began to show negative inotropic effects at 3×10^{-5} M, and contracture occurred, with a reduction in cardiac contractility (Fig. 4(B)). Therefore, NAd administration did not show any essential, peak action. On the other hand, SCH showed PIE in a dose-dependent manner, and the contractility at the time of 10^{-4} M SCH administration was 50%, regarding that after 10^{-6} M NAd administration as 100% (Fig. 3). The actions gradually became more marked, reaching a maximum

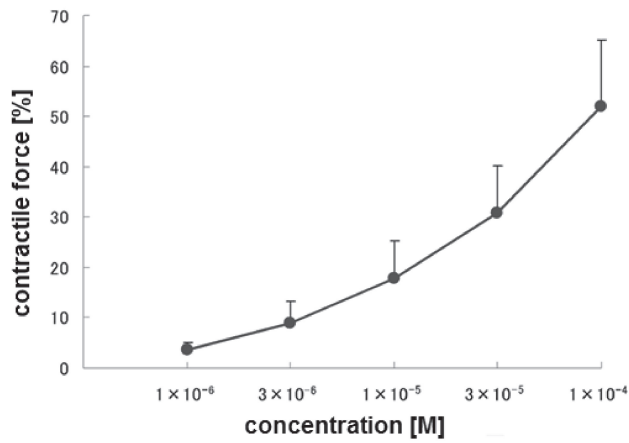


Fig. 3. PIE of SCH in right atrial specimens extirpated from guinea pigs

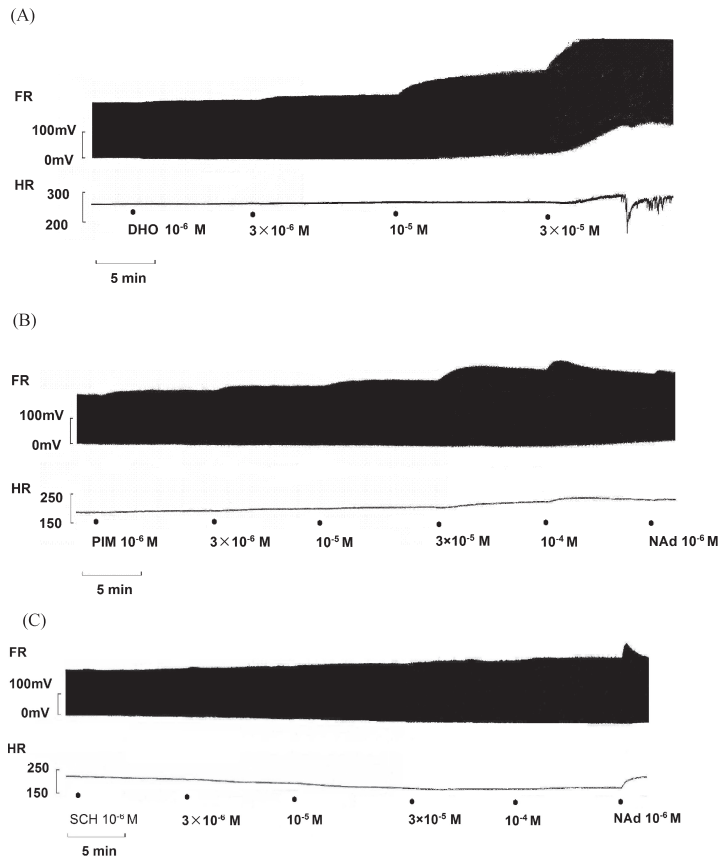
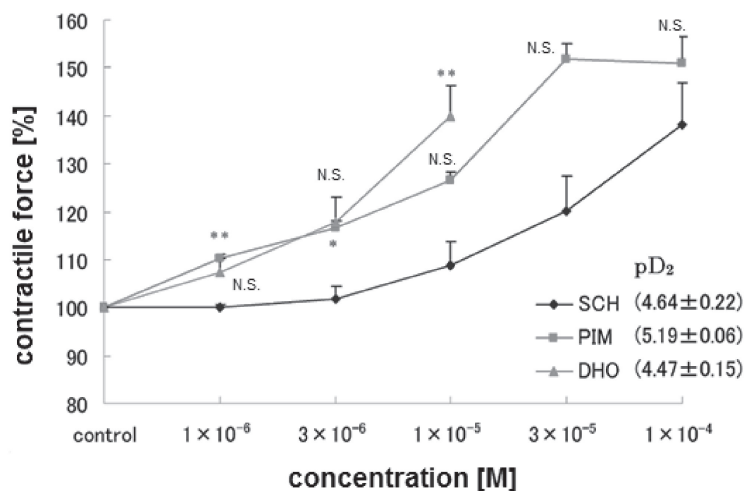


Fig. 4. Changes in the cardiac contractility and heart rate after the administration of DHO (A), PIM(B), or SCH(C) to the guinea-pig right atrium

(A)



(B)

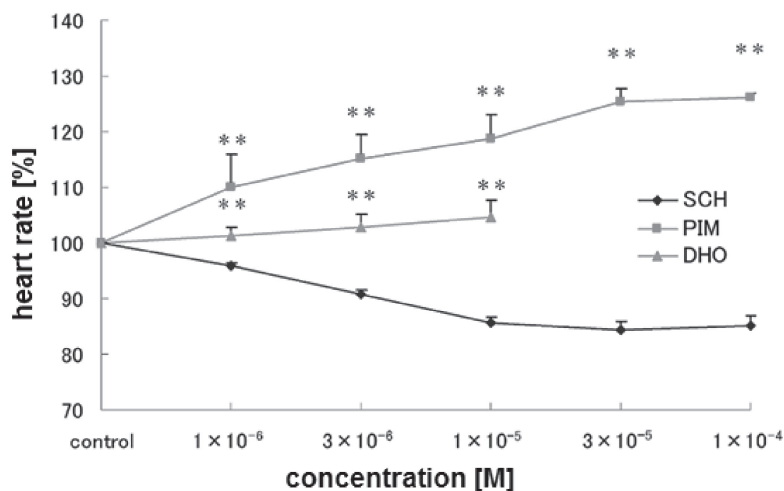


Fig. 5. Comparison of the effects of SCH, PIM, and DHO on the guinea-pig right atrium contractility (A) and heart rate (B)

Data are expressed as the mean values of 5 preparations \pm S. E. M; N. S.; * $p < 0.05$; ** $p < 0.01$ (SCH /S PIM, DHO)

after 10 minutes (Fig. 4(C)). The pD_2 values of PIM, SCH, and DHO were 5.19, 4.64, and 4.47, respectively.

As shown in Fig. 5(A), we compared cardiac contractility among SCH, PIM, and DHO. To compare the results between SCH and the other 2 compounds, the t-test was used. There were significant differences between SCH and PIM at 10^{-6} ($p < 0.01$) and 3×10^{-6} M ($p < 0.05$), but there were no significant differences at $\geq 10^{-5}$ M. There were no significant differences between SCH and DHO at

10^{-6} or 3×10^{-6} M, but there was a significant difference at 10^{-5} M ($p < 0.01$).

3.2 Actions on the heart rate

The 3 compounds showed different actions on the heart rate. As shown in Fig. 5(B), we compared the heart rate among SCH, PIM, and DHO. SCH slightly decreased the heart rate, whereas PIM increased it. After DHO administration, there were no changes. To compare the results between SCH and the other 2 compounds, the t-test was used. There were significant differences between SCH and PIM at 10^{-6} to 10^{-4} M ($p < 0.01$). There were also significant differences between SCH and DHO at 10^{-6} to 10^{-5} M ($p < 0.01$). SCH decreased the heart rate in a dose-dependent manner, and there was no increase even after 10 minutes at 10^{-4} M. Subsequently, the administration of 10^{-6} M NAd increased the heart rate. PIM markedly increased the heart rate. However, the administration of 10^{-6} M NAd after the appearance of contracture did not show any essential, peak action, although there was a slight increase in the heart rate. DHO slightly increased the heart rate, but the administration of 10^{-6} M NAd after the appearance of arrhythmia did not lead to recovery. Tadano et al. [6] reported that SCH showed PIE without influencing the heart rate, but there was an approximately 10% decrease 90 minutes after administration. In this study, the heart rate also reduced by 10%, which was consistent with the above finding. Cardiotonic glycosides also decrease the heart rate. In particular, they reduce oxygen consumption at the time of heart failure. Hayashi et al. [7] indicated that there was no influence of PIM on the heart rate in clinical practice. However, the results of this experiment showed that it increased the heart rate in comparison with the other drugs. As adverse reactions to PIM include tachycardia, PIM may act on the heart rate.

The changes in the LVDP and NO (TNO) signals in Langendorff specimens treated with SCH are presented in Fig. 6. The level of TNO increased in a dose-dependent manner.

We measured changes in the LVDP and TCa signals at the time of SCH/PIM administration in Langendorff specimens (Fig. 7).

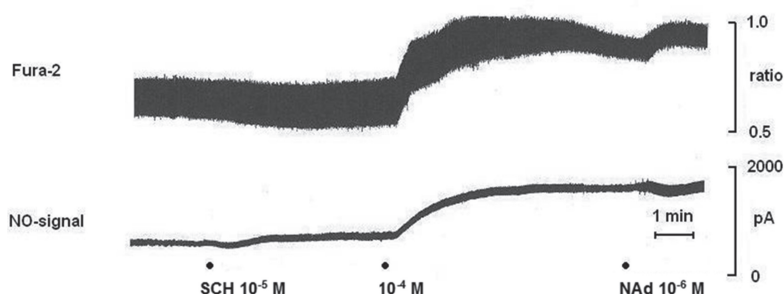


Fig. 6. SCH-related changes in transient Ca^{2+} (TCa) and NO (TNO) signals in normal Langendorff specimens

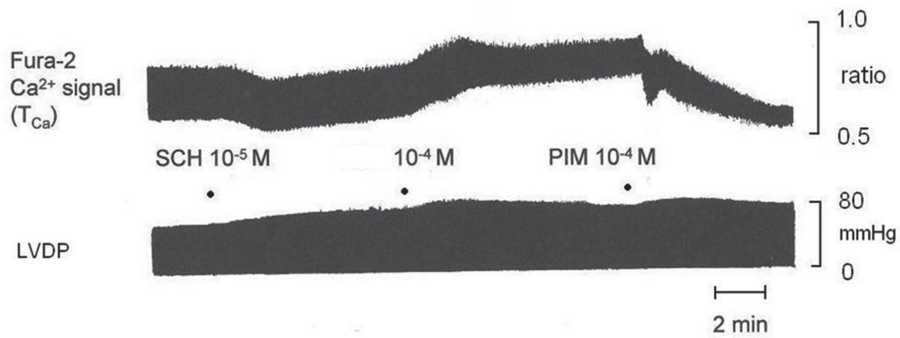


Fig. 7. SCH-/PIM-related changes in the LVDP and transient Ca^{2+} (T_{Ca}) signals in normal Langendorff specimens

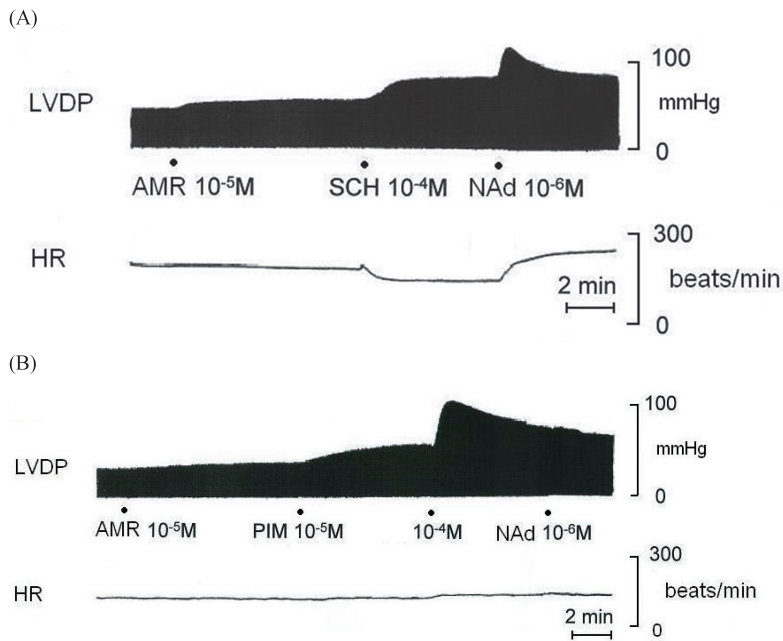


Fig. 8. PIE and PCE of SCH after pretreatment with a PDE inhibitor, amurionon (AMR) (A) and those of PIM after pretreatment with AMR (B)

After the administration of 10^{-5} M SCH, the level of T_{Ca} decreased with an increase in contractility, but it increased after 10^{-4} M SCH administration. Hotta et al. [8] reported the changes in the LVDP and T_{Ca}/NO (T_{NO}) signals after PIM administration in Langendorff specimens are shown in Fig. 8. 10^{-4} M PIM decreased the level of T_{Ca} despite an increase in contractility. PIM, as a drug for heart failure, which increases contractility through a contractile protein, slightly increased the level of T_{Ca} at 10^{-5} M , and decreased it at 10^{-4} M .

The PIE and PCE of SCH after pretreatment with a PDE inhibitor, amurinon (AMR) (A) and those of PIM after pretreatment with AMR (B) are presented in Fig. 8. 10^{-4} M SCH decreased the heart rate, whereas 10^{-4} M PIM slightly increased it (Fig. 8).

4. Discussion

Many studies have investigated Ca^{2+} sensitizers, considering that they may be potent cardiotonic agents from the perspective of energy efficiency in that they do not require energy necessary for Ca^{2+} transport in myocardial cells (activation energy). In addition, cardiotonic agents that promote Ca^{2+} mobilization, such as DHO, may induce excessive Ca^{2+} loading-related arrhythmia or myocardial cell disorder; therefore, Ca^{2+} sensitizers (PIM, SCH), which may not cause such disorders, may be useful for treating heart failure. The following 3 mechanisms by which Ca^{2+} sensitizers enhance Ca^{2+} sensitivity are assumed: ① enhancement of the Ca^{2+} -binding affinity of troponin C, ② promotion of interactions between the troponin-tropomyosin complex and actin, and ③ promotion of interactions between actin and myosin. Fujino et al. [9] indicated that PIM exhibited PIE by enhancing the Ca^{2+} sensitivity of troponin C in dog/guinea pig myocardial fibers. On the other hand, another study reported that PIM showed PIE by promoting interactions between the troponin-tropomyosin complex and actin [10]. Holubarsch et al. [11] verified that PIM enhanced contractility without changing the energy efficiency in papillary muscles extirpated from guinea pigs. Berger et al. [12] indicated that PIM increased the contractility of papillary muscles extirpated from guinea pigs in a dose-dependent manner. According to Tadano et al. [6], SCH enhanced Ca^{2+} sensitivity.

In this experiment, we compared the PIE of SCH with those of PIM and DHO using the t-test. There were no significant differences between SCH and PIM at $\geq 10^{-5}$ M. There was a significant difference between SCH and DHO at 10^{-5} M ($p < 0.01$). This suggests that the action mechanism of SCH involves the enhancement of Ca^{2+} sensitivity, as demonstrated for PIM. DHO exhibited PIE in a dose-dependent manner, but showed negative inotropic effects (NIE) at a high concentration (3×10^{-5} M), causing contracture. NAd administration after the onset of contracture did not lead to recovery. On the other hand, SCH exhibited PIE, as reported for PIM, but such actions further persisted after NAd administration; SCH may be more useful than PIM. DHO elevates the intracellular concentration of Na^+ by inhibiting Na^+/K^+ -ATPase, increasing Ca^{2+} influx from the extracellular area through the $\text{Na}^+/\text{Ca}^{2+}$ exchange system and exhibiting PIE through an increase in the intracellular concentration of Ca^{2+} . At $\geq 3 \times 10^{-5}$ M, DHO induced arrhythmia. This was possibly associated with further intracellular Ca^{2+} influx-related accumulation. NAd administration after the onset of arrhythmia did not lead to recovery. As SCH exhibited PIE without inducing intracellular Ca^{2+} accumulation, with the appearance of NAd actions, it may be more useful than DHO.

5. Conclusion

A Ca^{2+} sensitivity-enhancing drug, SCH, normally responded to NAd without causing arrhythmia. As SCH may not induce arrhythmia or myocardial cell disorder, it may be more useful than cardiotonic agents with intracellular Ca^{2+} concentration-increasing actions. In addition, a drug that does not markedly increase the heart rate may be useful for treating heart failure, as there is no increase in oxygen consumption, with no influence on the body.

SCH inhibits PDE III at approximately 65 μM [13], which is higher than the concentration of PIM at which such actions are observed (1 μM [2]). The inhibitory effects of SCH on PDE at a high concentration may be related to PIE. At a high concentration, Fura-2 Ca signals may increase.

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